

A Comparison of the Precipitation Technique and ImmunoCAP® FIEA for Measurement of IgG Antibodies to *Aspergillus fumigatus*

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Introduction

Aspergillus precipitins have traditionally been used to aid diagnosis and monitor treatment of aspergillosis. However, performing this assay can be time consuming with inter-operator variability. The new ImmunoCAP® fluorescent immuno enzyme assay (FIEA) produced by Phadia is a fast, automated method to detect specific IgG. For specific IgG *A. fumigatus* the manufacturer recommends that a level greater than 40mg/L is indicative of probable aspergillosis. This level has yet to be validated for individual patient groups and the clinical significance of levels is not known.

Aim

To compare *A. fumigatus* IgG levels obtained using the ImmunoCAP® FIEA test with the *A. fumigatus* precipitin test in patients with Cystic fibrosis (CF), Chronic Pulmonary Aspergillosis (CPA), and Allergic Bronchopulmonary Aspergillosis (ABPA).

Method

A blood sample was taken from 170 adult patients attending Manchester's National Aspergillosis Centre and 100 adult patients attending the Manchester Adult Cystic fibrosis Centre.

Serum was analysed by the Phadia ImmunoCAP® assay for specific IgG *A. fumigatus* and by counterimmunoelectrophoresis (CIE) for *A. fumigatus* precipitins. An ImmunoCAP® level >40mg/L was considered positive and 2-fold precipitin titres were recorded.

Patients were grouped into clinical disease categories:

- CPA patients were defined by radiology and serology as per Denning *et al* 2003 guidelines.
- Non CF ABPA patients were defined by Greenberger's essential criteria.
- CF patients were grouped into those with ABPA (2003 CF foundation consensus conference – minimum diagnostic criteria), sensitised (> Class 2 specific IgE *A. fumigatus* - ≥ 3.7 kUA/L), and controls (no evidence of ABPA and *A. fumigatus* IgE < 3.7 kUA/L).

Figure 1 ImmunoCAP® 250 machine

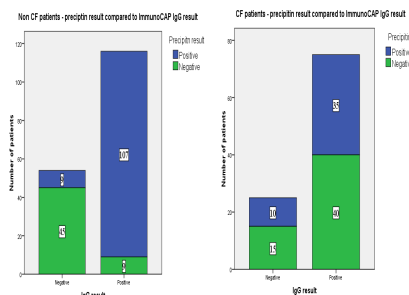


Figure 2 Positive precipitin test by CIE

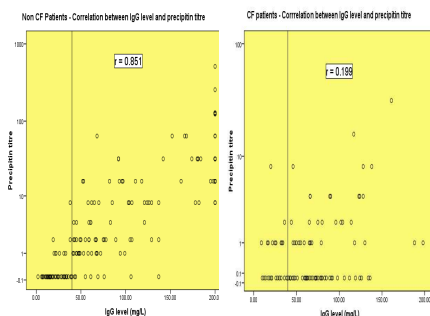


Results 1: Test Concordance

- In non-CF patients there was 92% concordance between a positive IgG result and a positive precipitin result.
- In contrast, for CF patients there was only 50% concordance between a positive IgG result and a positive precipitin result.



- Non-concordance in 38% of CF patients was due to a positive IgG but negative precipitin. A ROC curve, with an area under the curve of 0.593, demonstrated that raising the cut-off for a positive IgG would not improve concordance.
- There was a good correlation between IgG level and precipitin titre in the non-CF group ($r = 0.903$, $p < 0.01$) but this was not observed for the CF patients ($r = 0.199$, $p = 0.11$).



- The maximum level of ImmunoCAP® IgG reported was 200mg/L. Within this group precipitin titres varied widely from 1/8 to 1/512.

Results 2: Disease Category

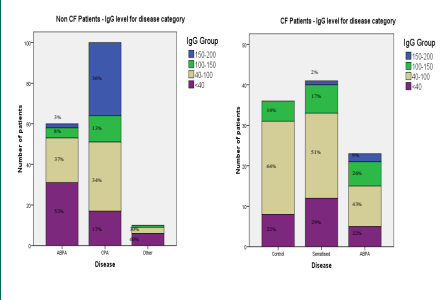
Table 1 Number of patients with a raised IgG (>40mg/L) and number with positive precipitins.

Disease (number of patients)	Percentage with IgG >40mg/L	Percentage with positive precipitin
CPA (100)	83%	84%
Non CF ABPA (60)	45%	45%
CF ABPA (23)	78%	57%
CF sensitised (41)	71%	41%
CF controls (36)	78%	42%

- Both IgG levels and precipitin titres are not good predictors of disease in CF and non CF ABPA patients.
- Patients with CPA had significantly greater IgG levels than all other disease categories.

Table 2 Mean IgG levels for disease categories

Disease	Mean IgG (mg/L)	SE (mg/L)
CPA	112	6.89
Non CF ABPA	49	5.47
CF ABPA	80	10.78
Sensitised	64	5.54
CF Control	67	5.74



Result 3: Monitoring Disease

Sequential monitoring in 10 CPA patients showed:

- Stable IgG and precipitin results in 6 patients.
- In 2 patients a rise in IgG also led to an increase in precipitin titre.
- In the last 2 patients IgG remained >200mg/L but precipitin titre fell from 1/128 to 1/32 and 1/128 to 1/64 respectively.

Conclusions

- In CF patients there is little correlation between the IgG ImmunoCAP® level and the precipitin titre.
- Neither the IgG ImmunoCAP® level or the precipitin titre correlates to *Aspergillus* disease category in CF.
- In non CF ABPA there is a good correlation between tests but neither is a good marker of disease presence.
- The IgG ImmunoCAP® may be a useful alternative to the *Aspergillus* precipitin test in patients with CPA. It can support diagnosis and monitoring treatment for patients with CPA. However once values reach >200mg/L precipitins are more informative of disease activity.

References

- Greenberger PA. Allergic Bronchopulmonary Aspergillosis. *J Allergy Clin Immunol.* Nov 2002;110(5):685-692.
- Stevens DA *et al.* Allergic bronchopulmonary aspergillosis in cystic fibrosis-state of the art: Cystic Fibrosis Foundation Consensus Conference. *Clin Infect Dis.* 2003. 37 Suppl 3: p. S225-64.
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